

FERMENTATION MEDIUM : *NITROGEN SOURCE AND OTHER CONSTITUENTS*

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NITROGEN SOURCE

- Most industrial microbes can utilize both inorganic and organic nitrogen sources. Inorganic nitrogen may be supplied as ammonium salts, often ammonium sulphate and diammonium hydrogen phosphate, or ammonia.
- Organic nitrogen sources include amino acids, proteins and urea.
- Nitrogen is often supplied in crude forms that are essentially byproducts of other industries, such as corn steep liquor, yeast extracts, peptones and soya meal.

CORN STEEP LIQUOR

- Corn steep liquor is a byproduct of starch extraction from maize and its first use in fermentations was for penicillin production in the 1940s.
- The exact composition of the liquor varies depending on the quality of the maize and the processing conditions.
- Concentrated extracts generally contain about 4% (w/v) nitrogen, including a wide range of amino acids, along with vitamins and minerals.

YEAST EXTRACTS

- Yeast extracts may be produced from waste baker's and brewer's yeast, or other strains of *S. cerevisiae*.
- Extracts are available as liquids containing 50–65% solids, viscous pastes or dry powders.

Total proteins, peptides & amino acids (% w/v)	73–75
free amino acids	35–40
peptides less than 600 Da	10–15
material above 600 Da	20–30
Vitamins (µg/g)	
thiamin	30
riboflavin	120
niacin	700
pyridoxine	20
folic acid	30
calcium pantothenate	300
biotin	2.5

Note: mineral content varies with the processing steps used.

PEPTONES

- Peptones are usually too expensive for large-scale industrial fermentations.
- They are prepared by acid or enzyme hydrolysis of high protein materials: meat, casein, gelatin, keratin, peanuts, soy meal, cotton seeds, etc.
- Their amino acid compositions vary depending upon the original protein source.
- For example, gelatin derived peptones are rich in proline and hydroxyproline, but are almost devoid of sulphur-containing amino acids; whereas keratin peptone is rich in both proline and cystine, but lacks lysine.

SOYA BEAN MEAL

- Residues remaining after soya beans have been processed to extract the bulk of their oil are composed of 50% protein, 8% non-protein nitrogenous compounds, 30% carbohydrates and 1% oil.
- This residual soya meal is used in antibiotic fermentations.

MINERALS

- Normally, sufficient quantities of cobalt, copper, iron, manganese, molybdenum, and zinc are present in the water supplies, and as impurities in other media ingredients.
- For example, corn steep liquor contains a wide range of minerals that will usually satisfy the minor and trace mineral needs.
- Occasionally, levels of calcium, magnesium, phosphorus, potassium, sulphur and chloride ions are too low to fulfil requirements and these may be added as specific salts.

VITAMINS AND GROWTH FACTORS

- Vitamins are growth factors which fulfill specific catalytic needs in biosynthesis and are required in only small amounts.
- They are organic compounds that function as coenzymes or parts of coenzymes to catalyze many reactions.
- The vitamins most frequently required are thiamin and biotin. Required in the greatest amounts are usually niacin, pantothenate, riboflavin, and some (folic derivatives, biotin, vitamin B₁₂ and lipoic acid) are required in smaller amounts.

PRECURSORS

- Some fermentations must be supplemented with specific precursors, notably for secondary metabolite production.
- When required, they are often added in controlled quantities and in a relatively pure form.
- Examples include phenylacetic acid or phenylacetamide added as side-chain precursors in penicillin production.
- Dthreonine is used as a precursor in l-iso-leucine production by *Serratia marsescens*,

INDUCERS AND ELICITORS

- If product formation is dependent upon the presence of a specific inducer compound or a structural analogue, it must be incorporated into the culture medium or added at a specific point during the fermentation.
- In plant cell culture the production of secondary metabolites, such as flavonoids and terpenoids, can be triggered by adding elicitors.

INHIBITORS

- Inhibitors are used to redirect metabolism towards the target product and reduce formation of other metabolic intermediates.

CELL PERMEABILITY MODIFIERS

- These compounds increase cell permeability by modifying cell walls and/or membranes, promoting the release of intracellular products into the fermentation medium.
- Compounds used for this purpose include penicillins and surfactants. They are frequently added to amino acid fermentations, including processes for producing l-glutamic acid using members of the genera *Corynebacterium* and *Brevibacterium*

ANTI-FOAMS

- Antifoams are necessary to reduce foam formation during fermentation.
- Foaming is largely due to media proteins.
- If uncontrolled the foam may block air filters, resulting in the loss of aseptic conditions; the fermenter becomes contaminated and microorganisms are released into the environment.
- Natural antifoams include plant oils (e.g. from soya, sunflower and rapeseed), deodorized fish oil, mineral oils and tallow. The synthetic antifoams are mostly silicon oils, poly alcohols and alkylated glycols.



Thank you